

Developmental Morphology of Limb Reduction in *Hemiergis* (Squamata: Scincidae): Chondrogenesis, Osteogenesis, and Heterochrony

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ABSTRACT Digit loss is a common theme in tetrapod evolution that may involve changes in several developmental processes. The skink genus *Hemiergis* provides an ideal model to study these processes in closely related taxa: within three Western Australian *Hemiergis* species, digit quantity ranges between two and five. For three consecutive reproductive seasons, gravid females of *Hemiergis* were collected in the field and their embryos prepared for histological analysis of limb skeletal development (chondrogenesis and osteogenesis). Comparative studies of skeletal developmental morphology demonstrate that limbs with fewer than five digits do not result from a simple truncation of a putative ancestral (five-digit) developmental program. The developmental and adult morphologies in two-, three-, and four-digit *Hemiergis* are neither predicted nor explained by a simple model of heterochrony involving either chondrogenesis or osteogenesis. In postnatal *Hemiergis*, digit number and relative limb length do not correlate in a simple linear fashion. Instead, limb size and digit reduction may correlate with substrate conditions and burrowing behavior. *J. Morphol.* 254:211–231, 2002. © 2002 Wiley-Liss, Inc.

KEY WORDS: *Hemiergis*; skink; limb development; limb reduction; digit; chondrogenesis; osteogenesis; heterochrony

Digit loss is a common theme in the evolution of many extant and extinct tetrapod groups (Sewertzoff, 1931; Gans, 1975; Alberch and Gale, 1985; Greer, 1991; Carroll, 1996). Among squamates, limb reduction—defined as the phylogenetic loss of one or more limb skeleton elements relative to an ancestral morphology (Fig. 1)—is especially frequent, occurring independently 62 times in 53 lineages (Greer, 1991). In skinks alone, reductions have occurred 31 times in 25 lineages (Greer, 1991).

Lizards comprise a highly diverse group of organisms, yet the patterns of digit loss within this group are remarkably conservative across taxa (and among tetrapods in general; Morse, 1872; Romer, 1966). In general, digits are lost in the following order: I > V > II > III > IV (Sewertzoff, 1931; Greer, 1987, 1989, 1991). This conservatism may be rooted in convergent locomotor adaptations, shared developmental constraints, or perhaps both. For example, Greer (1991) proposes that evolutionary stability of

the central digits may be correlated with aspects of autopod function: digits III and IV may be the most important in locomotion and thus selection favors their retention.

Developmental factors may play a major role in patterns of limb reduction as well. Amniote limb development programs appear to be highly conserved (Burke and Alberch, 1985; Shubin and Alberch, 1986; Shubin, 1991), which may severely constrain the potential range of adult morphologies. In general, limb skeletal condensations first appear proximally and yield other condensations through segmentation and bifurcation events. Most of these events occur along the posterior side of the limb, through the “primary axis” of condensation connectivity that includes the ulna (fibula in the hind limb), ulnare (calcaneum), distal carpal (tarsal) 4, and digit IV (Fig. 1; Burke and Alberch, 1985; Shubin and Alberch, 1986). This axis then curves anteriorly and sequentially yields digits III, II, and I (the “digital arch”). Burke and Alberch (1985) and Shubin and Alberch (1986) regard digit V as a neomorphic structure that does not originate from the primary axis or digital arch. Alberch and Gale (1985) demonstrated that patterns of diversity in amphibian limbs depend largely on the developmental properties that characterize specific groups. Similarly, permutations of adult digit configurations in amniote limbs may be subject to developmental constraints imposed by the highly conserved primary axis and digital arch pattern of chondrogenesis

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(Holder, 1983; M.D. Shapiro and N.H. Shubin, unpubl. obs.).

Despite a wealth of information on adult morphology in reduced-limbed squamates (Cope, 1892; Presch, 1975; Papenfuss, 1982; Choquenot and Greer, 1989; Greer, 1987, 1989, 1990, 1991; Tchernov et al., 2000, and references therein), the developmental origins of squamate limb reduction remain poorly understood. Developmental modes of digit loss in squamates may include evolutionary changes in ontogenetic rates or sequences. These changes can produce heterochronies, broadly defined as phyletic changes in morphology resulting from alterations of ancestral developmental timing (De Beer, 1930; Gould, 1977, 2000; Alberch et al., 1979). Several authors implicate truncation of an ancestral developmental program—"progenesis" in the terminology of heterochrony (Alberch et al., 1979)—as a mode of digit loss among squamates (e.g., Essex, 1927; Gans, 1975; Greer, 1987: "partial truncations"), resulting in pedomorphic descendants (Müller, 1991). Other observations of adult reduced-limbed morphologies and general amniote limb development, however, predict that many limb configurations with fewer than five digits do not result from the simple truncation of ancestral pentadactyl ontogenetic trajectories (e.g., Shubin and Alberch, 1986; Greer, 1987, 1991). Nevertheless, neither hypothesis has been tested in a developmental framework.

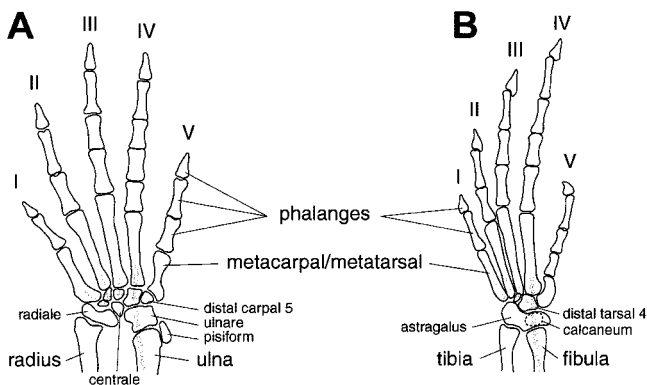


Fig. 1. Generalized manus and pes skeletons and primary axis of limb development in lizards. **A**: Distal forelimb skeleton of a lizard, beginning proximally with the distal ends of the ulna and radius, followed distally by the carpals and digits. Digits are numbered from anterior (digit I) to posterior (digit V). The primitive lizard phalangeal formula (number of phalanges per digit, beginning with the anterior digit) is 2-3-4-5-3 for the forelimb (Romer, 1956). The primary axis of limb development (heavy shaded line) runs through the posterior elements of the limb and digit IV (Burke and Alberch, 1985; Shubin and Alberch, 1986). This axis subsequently curves anteriorly and, through a series of bifurcations and segmentations (narrow shaded lines), yields the anterior digits. **B**: Distal hind limb skeleton, beginning proximally with the distal tibia and fibula, followed by the tarsals and digits. The primitive lizard hind limb phalangeal formula is 2-3-4-5-4 (Romer, 1956). The dashed oval in **B** indicates the approximate position of the embryonic calcaneum before fusion with the astragalus. Drawings are based on multiple specimens of the skink *Glaphyromorphus gracilipes* and are not to scale.

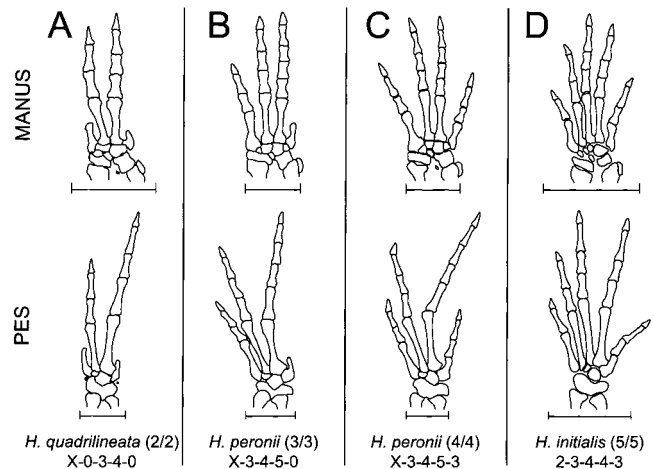


Fig. 2. Adult manus and pes morphologies of **(A)** *Hemiergis quadrilineata* (2/2), **(B)** *H. peronii* (3/3), **(C)** *H. peronii* (4/4), and **(D)** *H. initialis* (5/5). For each morph, the manus is in the top row, pes in the bottom row. Anterior is to the left and distal is up in all panels. Phalangeal formulae are listed as described in Figure 1. Scale bars = 1 mm.

The Australian skink genus *Hemiergis* provides a rare chance to test these hypotheses in the context of closely related species. Limb reduction is common among Australian skinks and *Hemiergis* represents the best example of graded digit loss among humus-dwellers (Choquenot and Greer, 1989; Greer, 1989, 1991). Within three closely related coastal Western Australian (WA) representatives, *Hemiergis* features a range of between two and five digits on the fore- and hind limbs. *Hemiergis quadrilineata* is a two-digit morph (two fingers / two toes, or 2/2), and *H. initialis* (5/5) has five digits (Fig. 2A,D). A third species, *H. peronii*, comprises two parapatric populations with fixed digit numbers: a three-digit western coastal population (*H. peronii*, 3/3) and a four-digit population (*H. peronii*, 4/4) that inhabits the southern coast and inland forests (Choquenot and Greer, 1989) (Figs. 2B,C, 3).

A principal aim of this study is to describe comparative patterns of limb skeletal chondrogenesis and osteogenesis in *Hemiergis* species and populations with different numbers of digits (hereafter referred to as different "morphs"). This study of developmental morphology in *Hemiergis* will consider several questions about digit loss not approachable in distantly related traditional model species of tetrapod development (i.e., the mouse *Mus musculus*, the chicken *Gallus domesticus*, and the anuran *Xenopus laevis*). First, what differences in early skeletal ontogeny yield different numbers of digits in species of a single genus? To address this question, I present a detailed examination of chondrogenesis and osteogenesis in *Hemiergis* through serial sectioning and whole-mount clearing and staining of cartilage and bone. Second, based on these chondrogenesis and ossification data, does heterochrony

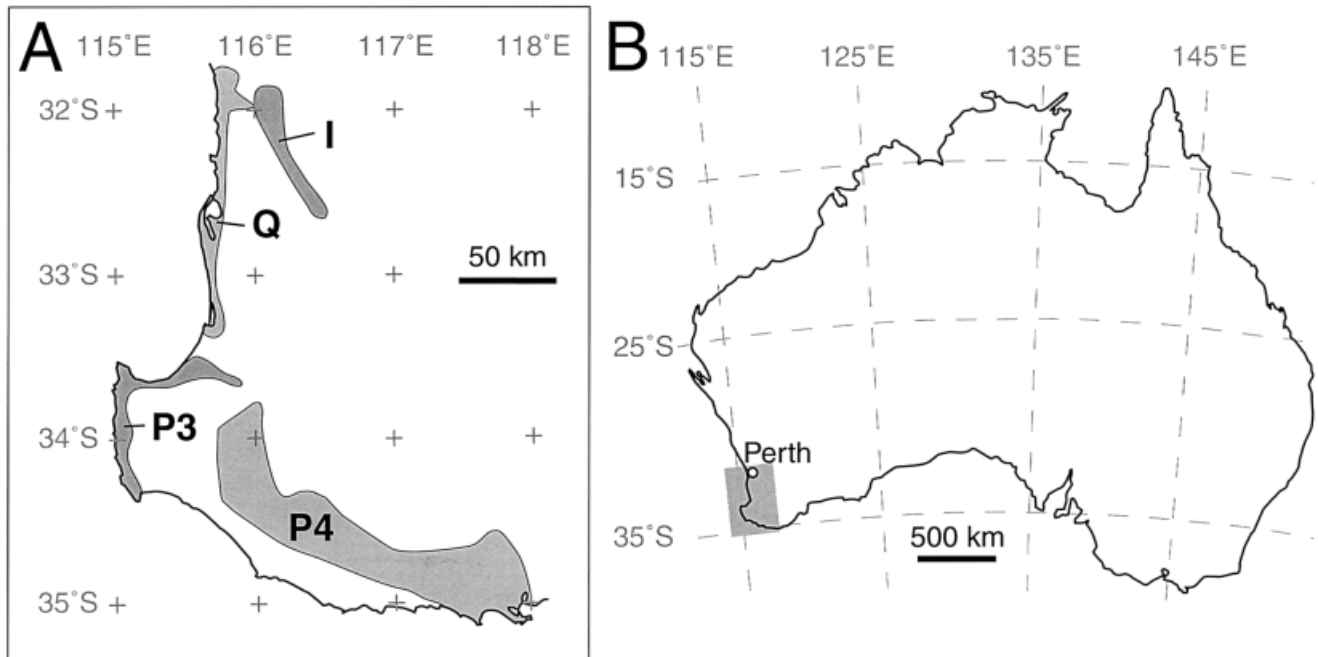


Fig. 3. Map of localities sampled in this study. **A:** Map of southwest corner of Western Australia with collection areas for *Hemiergis quadrilineata* (2/2), Q; *H. peronii* (3/3), P3; *H. peronii* (4/4), P4; and *H. initialis* (5/5), I. **B:** Map of mainland Australia. The shaded rectangle represents the enlarged area in **A**.

have any predictive or explanatory power in the context of limb reduction in *Hemiergis*? Heterochrony previously has been implicated in squamate digit loss (see above), but such hypotheses have never been tested directly. Third, what are the positional homologies of the digits in different limb configurations of *Hemiergis*? Adult digit homologies are easily discernible when five digits are present (as in *H. initialis*, 5/5), but not when one or more digits are missing (as in *H. peronii*, 3/3 and 4/4; and *H. quadrilineata*, 2/2). Studies of early digit development can help rectify this uncertainty. Fourth, in postnatal specimens, what is the relationship between digit number and relative limb length in *Hemiergis*? That is, do morphs with fewer digits also have shorter limbs relative to their body lengths? While instances of limb element loss and limb shortening have been documented extensively (see Greer, 1990; Gans, 1975, for reviews) the relationship between the two (i.e., whether limbs with fewer digits are also typically shortened) is poorly understood. Multiple regression analyses of limb and body lengths in *Hemiergis* will be used to test the hypothesis that digit loss is correlated with a decrease in overall limb size.

MATERIALS AND METHODS

Specimen Collection

Hemiergis quadrilineata (2/2), *H. peronii* (3/3 and 4/4), and *H. initialis* (5/5) are live-bearing, semifossorial species that inhabit

the semihumid to humid coastal shrublands and woodlands of southwestern WA (Fig. 3; an additional species not considered in this study inhabits a restricted, arid, inland region) (Choquet and Greer, 1989; Greer, 1989; Storr et al., 1999; Cogger, 2000). Gravid *Hemiergis* females were wild-caught between 1997 and 2000; collecting expeditions began in October or November of each year and ended in January of the next. All animals were collected by hand in leaf litter or under logs, rocks, sheet metal, and other debris. Captured animals were transported to the Western Australian Museum (WAM; Perth, Australia) and were either sacrificed within 24 h for embryo processing or were held in captivity in glass aquaria lined with 2–8 cm of moist sand and leaf debris. Captive gravid lizards that were not sacrificed by the end of each expedition were transported alive to the Museum of Comparative Zoology (MCZ; Cambridge, MA) to allow further embryo development and harvesting. While in captivity, lizards subsisted on a diet of termites and small mealworms at the WAM or pinhead crickets and mealworms at the MCZ.

Embryo Harvesting and Fixation

For embryo harvesting, gravid females were sacrificed and a longitudinal incision was made along the ventral abdomen, beginning just posterior to the sternum and extending to the anterior tips of the pubes. Oviducts containing eggs were removed and submerged in either phosphate-buffered saline (PBS) or 0.4% aqueous sodium chloride for dissection. Embryos were removed from their eggs, staged (Dufaure and Hubert, 1961; translated by Porter, 1972), fixed overnight at 4°C in either Dent fixative (20% dimethyl sulfoxide in methanol; Dent et al., 1989) or 4% paraformaldehyde in PBS, then dehydrated through a graded methanol series and stored in 100% methanol at –20°C. Some late (stage 36 and higher) embryos were fixed in 10% neutral-buffered formalin (NBF) and stored in 70% ethanol to optimize bone staining. Adult specimens were fixed in 10% NBF and stored in 70% ethanol.

TABLE 1. (Continued.)

Stage	Specimen	SVL	H	R	U	re	ue	c	Phalanges															Fusions										
									Distal carpals					Metacarpals					I II III IV V															
									1	2	3	4	5	1	2	3	4	5	1	1	1	1	1		2	2	2	2	2	3	3	3	3	3
35/36	10922	NA	2	2	2	2	2	1	0	1	1	2	0	2	2	2	2	2	2	2	2	2	0	2	2	2	2	0	1	1	0	0	0	—
35	10496	9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	2	2	1	2	2	0	0	0	—
36+	10935	13	2	2	2	2	2	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—
37	38153	13	3?	3?	3?	2	2	2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—
38	38155	14	3?	3?	3?	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—
39	10945	13	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—
40	10944	16	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	dc4-dc5
J	HI-001	18	4	3	3	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	
J	HI-104	20.5	3	3	3	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	
J	HI-102	21	3	3	3	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
J	HI-103	22	3	3	3	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
J	HI-002	20	3	3	3	2	3	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
J	HI-006	20.5	4	3	4	3	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
J	HI-003	21	3	3	3	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
J	HI-105	19	3	3	3	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
J	HI-004	20	3	3	3	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
J	HI-101	19.5	4	3	3	3	2	0	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	—	dc4-dc5	
S	HI-201	28.5	4	4	4	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	—	dc4-dc5	
S	HI-202	30	4	4	4	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	—	dc4-dc5	
A	HI-302	30	4	4	4	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	—	dc4-dc5	
A	HI-301	40	4	4	4	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	—	dc4-dc5	

For each element, 0 = absent, 1 = prechondrogenic, 2 = chondrogenic, 3 = ossified, and 4 = secondary centers of ossification present (long bones only); question marks (?) after a number indicate ambiguous staining; elements not normally present in a given morph are indicated with dashes (—); snout–vent length (SVL) is rounded to the nearest millimeter for most embryonic specimens; all 5-digit specimen numbers are “MCZ A” series. J, juvenile; S, subadult; A, adult; H, humerus; R, radius; U, ulna; re, radiale; ue, ulnare; c, centrale; dc, distal carpal; phalanges are listed by digit number and proximodistal position (e.g., II-3 is the third phalanx of the second digit).

Clearing and Staining of Cartilage and Bone

Developmental series from each morph were cleared and stained as whole mounts using a modification of a standard technique to visualize chondrogenesis and ossification patterns (Dingerkus and Uhler, 1977). Modifications included a lower trypsin concentration (0.10–0.25%) for overnight treatment for small embryos (stage 34 and earlier) and embryo bleaching (when necessary) in 1 µl/ml hydrogen peroxide in 25% glycerol (in 0.5% aqueous potassium hydroxide). A dark alizarin red stain was achieved using a 0.001–0.002% solution in 2% potassium hydroxide.

Skeletal condensations that stained with Alcian blue were scored as present and prechondrogenic tissues were scored separately; structures composed of the latter were very faint and lacked the typical chondrocyte morphology visible in whole mount at high magnification (though it is possible that some of these elements were indeed very early chondrogenic). Whole-mount observations were occasionally verified through stained serial sections (Hall, 1986; Celestine blue step omitted).

Limb skeleton elements stained with alizarin red were scored as ossified and secondary (epiphyseal) centers of long bone ossification were scored if red stain localized within the cartilaginous epiphyses. Advanced embryos often did not stain successfully with alizarin red; most of these specimens were either fixed in Dent fixative or stored for an extended time in methanol. In these instances, unstained bones were scored as ossified if bone was clearly visible through microscopy. Clearing and staining of postnatal specimens was performed as described (Dingerkus and Uhler, 1977) but with the alizarin red staining modifications noted above.

Regression Analysis of Limb Lengths

Differences in postnatal limb lengths between the four *Hemiergis* morphs were assessed by multiple regression analysis control-

ling for snout-vent length (SVL). Specimens from the 1999–2000 collecting expedition were measured for SVL and limb length—defined here as the measurement from the body wall to the tip of digit IV normal to the body axis—at the time of sacrifice (i.e., before fixation). Left limbs were measured unless they were missing or incomplete. Only female adults were measured, but neonates and juveniles of both sexes were measured. A total of 20 *H. quadrilineata* (2/2; SVL range 30.35–69.28 mm), 66 *H. peronii* (3/3; 26.10–62.61 mm), 77 *H. peronii* (4/4; 19.84–67.26 mm), and 51 *H. initialis* (5/5; 29.25–49.57 mm) were measured. Fore- or hind limb length was used as the dependent variable for each analysis and SVL and three dummy variables (*H. initialis*, 5/5, and the two *H. peronii* morphs) were used as independent variables. *Hemiergis quadrilineata* (2/2) was used as the default variable.

RESULTS

Condensation of the Limb Skeletons

Stylopod and zeugopod. Limb element condensations and ossifications are summarized in Tables 1 and 2. Early stages of *Hemiergis* limb development closely resemble known ontogenetic sequences of pentadactyl lizards (Sewertzoff, 1904, 1931; Mathur and Goel, 1976) and amniotes in general (Holmgren, 1933; Burke and Alberch, 1985; Shubin and Alberch, 1986) (Fig. 4). Condensations appear from proximal to distal, beginning with the stylopod elements (forelimb: humerus; hind limb: femur), which are present as cartilage by stage 31. The zeugopod elements (forelimb: radius and ulna; hind limb: tibia and fibula) condense and chondrify by the end of stage 32. In contrast with the branching “Y” pattern of chon-

TABLE 2. (Continued.)

Stage	Specimen	SVL	Fe	T	Fi	as	ca	Phalanges																				Fusions										
								dt's		Metatarsals					I II III IV V I II III IV V II III IV V III IV IV																							
								3	4	1	2	3	4	5	1	1	1	1	1	2	2	2	2	2	3	3	3		3	4	4	5						
35/36	10922	NA	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	2	2	0	1	1	0	0	0	—	as-ca	
35	10496	9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	2	2	2	2	1	2	2	0	0	0	—	as-ca
36+	10935	13	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
37	38153	13	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
38	38155	14	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
39	10945	13	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
40	10944	16	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-001	18	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-105	19	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-102	21	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-006	20.5	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-101	19.5	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-103	22	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-104	20	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-004	20	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-002	20	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
J	HI-003	21	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	—	as-ca	
S	HI-201	28.5	4	4	4	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	—	as-ca	
S	HI-202	30	4	4	4	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	—	as-ca	
S	HI-302	30	4	4	4	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	—	as-ca	

Fe, femur; T, tibia; Fi, fibula; as, astragalus; ca, calcaneum; dt, distal tarsal; numbering system and phalangeal abbreviations as for Table 1.

drogenesis observed in some tetrapods (Burke and Alberch, 1985; Shubin and Alberch, 1986), the zeugopod elements of *Hemiergis* condense separately.

Carpals. Mesopodial development along the amniote primary axis is characterized by bifurcation and segmentation events, whereas anterior ele-

ments (i.e., from the radius) exhibit segmentation only (Shubin and Alberch, 1986; Müller, 1991). In the forelimb of *Hemiergis*, the ulnare (ue), centrale (c), and distal carpals (dc) 1–4 originate from the primary axis; the radiale (re) and dc5 likely do not. In several specimens, two faint condensations were visible in the re position early in development (also noted by Müller, 1991). Table 3 outlines the orders of carpal appearance in the different *Hemiergis* morphs.

Other skeletal elements of the hand include the pisiform (pis), which is likely a sesamoid bone that does not originate from a segmentation or bifurcation event (Shubin and Alberch, 1986; Rieppel, 1992a). An additional small cartilage appears between the distal radius and ulna at stage 38 or later in some specimens and is ossified in some adults (e.g., Fig. 2A,C). The position occupied by this small bone suggests that it is the intermedium, but its late appearance suggests that it may be a sesamoid element. The latter scenario would be supported if the element lies within a tendon, but this possibility has not been pursued.

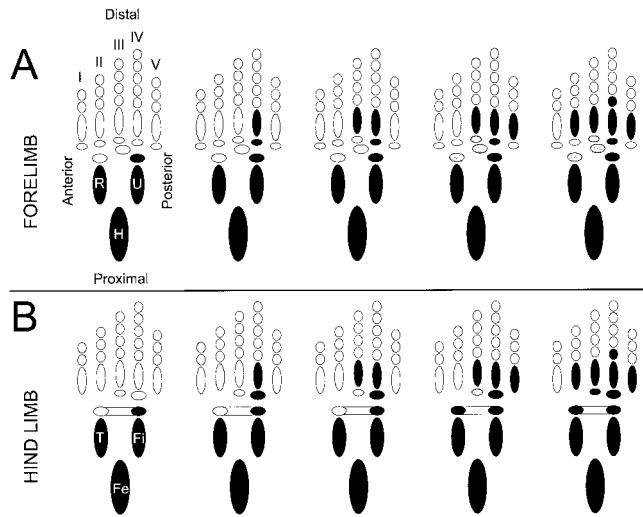


Fig. 4. Shared limb skeleton condensation configurations in *Hemiergis*. In this schematic diagram, elements present in all morphs at a given stage appear as blackened shapes, elements exhibiting variability appear in gray. Younger stages are to the left of the figure, older ones are to the right. **A:** Forelimb condensations first appear proximally and include the humerus, radius, and ulna. Digits II–V appear in the order, IV > III > V > II. Phalangeal condensation begins with digit IV. **B:** Hind limb elements appear in a similar sequence, beginning with the femur, tibia, and fibula. Fe, femur; Fi, fibula; H, humerus; R, radius; T, tibia; U, ulna; digits I–V are indicated by roman numerals.

TABLE 3. Carpal condensation sequences in *Hemiergis*

Species	Order of condensation appearance
<i>H. quadrilineata</i> (2/2)	ue > dc4 > dc3 ≥ (c, re) > (dc5, dc2) > pis
<i>H. peronii</i> (3/3)	ue > dc4 > dc3 > dc5 > c ≥ re > dc2 > pis
<i>H. peronii</i> (4/4)	ue > dc4 > dc3 > c ≥ re > dc5 > dc2 > pis
<i>H. initialis</i> (5/5)	ue > dc4 > c ≥ (re, dc3) > dc2 > (dc1, dc5) > pis

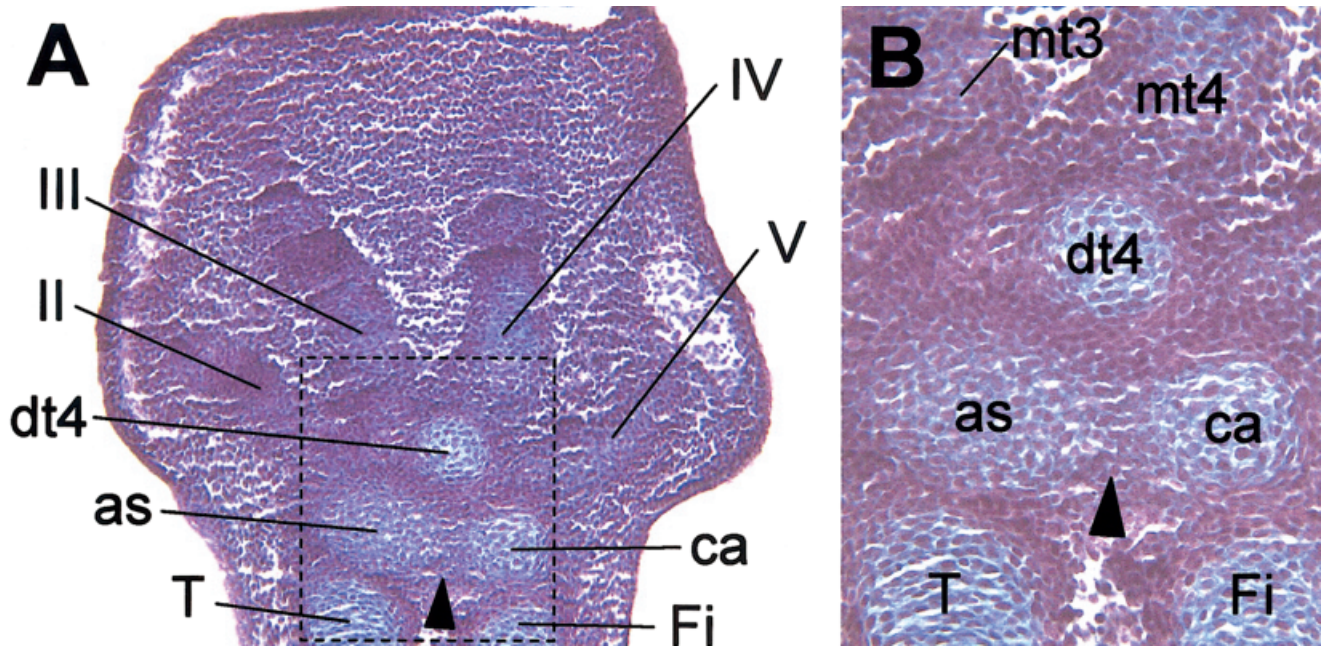


Fig. 5. Coronal section of a stage 33 *Hemiergis quadrilineata* (2/2) (MCZ A10422) hind limb showing incipient fusion of the astragalus and calcaneum. **A**: Fusion begins with mesenchymal connectivity (arrowhead), followed by chondrogenesis at later stages (magnification = 20 \times). No mesenchymal connectivity between the as and ca is observed until this stage. **B**: Magnification of boxed area in **A**, 50 \times . as, astragalus; ca, calcaneum; dt, distal tarsal; Fi, fibula; mt, metatarsal; T, tibia; digits II–V indicated by roman numerals in **A**.

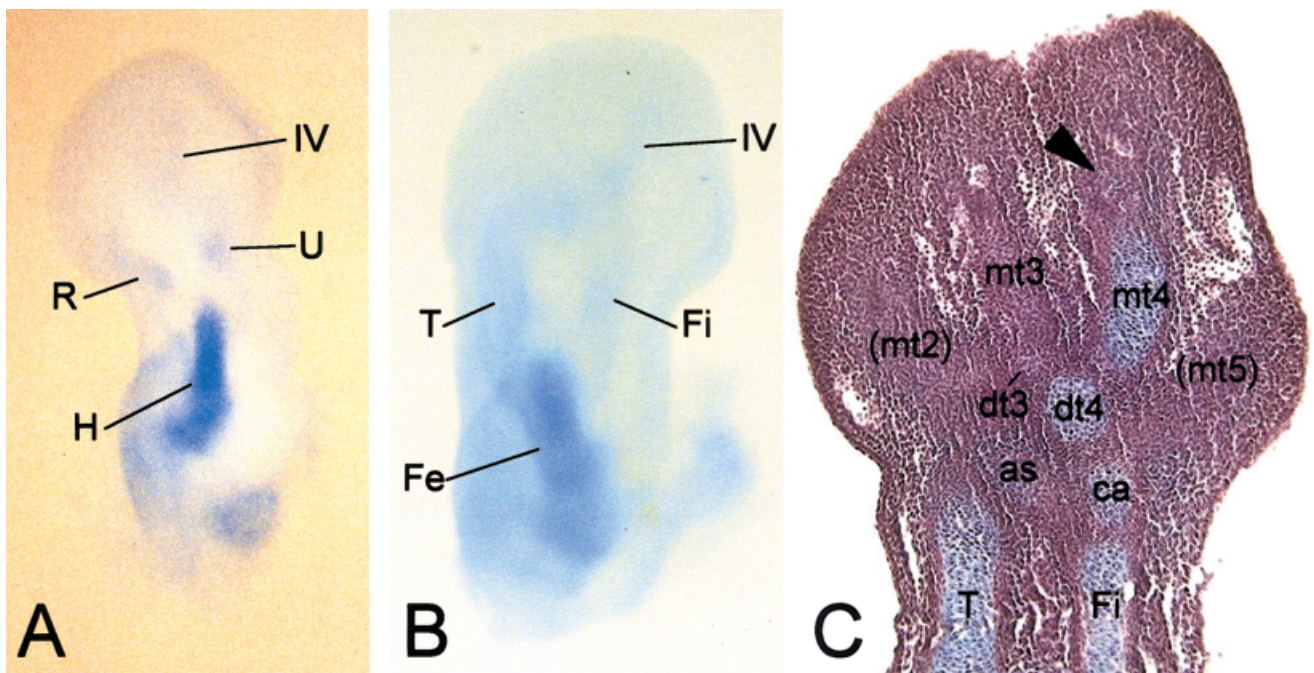


Fig. 6. Skeletal condensations along the primary axis in *Hemiergis* at stages 32 and 33. **A,B**: Stage 32 forelimb (**A**) and hind limb (**B**) of *H. peronii* (4/4) (MCZ A38061) cleared and stained with Alcian blue. Cartilaginous elements appear in the stylopodia and zeugopodia of both limbs and faint autopodial condensations representing presumptive digit IV are also visible in both limbs. Serial sections of similarly aged limbs reveal only mesenchymal condensations in the autopod (not shown), and hence the digits in **A** and **B** are likely not cartilaginous. **C**: Coronal section of a stage 33 *H. peronii* (4/4) (MCZ A38073) hind limb. The primary axis of limb skeletal condensation passes through the fibula, calcaneum, distal tarsal 4, and metatarsal 4. The first phalangeal condensation to appear in the fore- and hind limbs of all *Hemiergis* morphs studied is the proximal phalanx of digit IV (arrowhead). Only mesenchymal portions of metatarsals 2 and 5 are visible in this section. as, astragalus; ca, calcaneum; dt, distal tarsal; Fe, femur; Fi, fibula; H, humerus; IV, digit IV condensation; mt, metatarsal; R, radius; T, tibia; U, ulna.

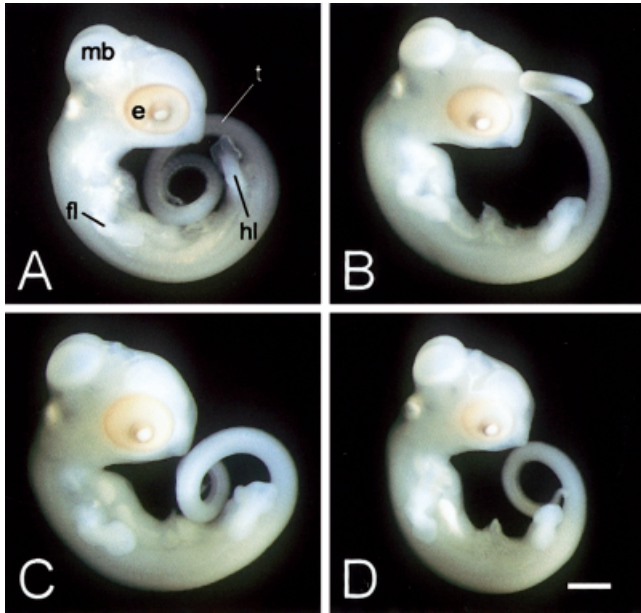


Fig. 7. Stage 33 embryos of *Hemiergis*. Embryos of (A) *H. quadrilineata* (2/2), (B) *H. peronii* (3/3), (C) *H. peronii* (4/4), and (D) *H. initialis* (5/5) have similar external morphologies at this and earlier stages. e, eye; fl, forelimb; hl, hind limb; mb, midbrain; t, tail. Scale bar = 1 mm.

Tarsals. Tarsal condensation sequences are more similar across species, perhaps owing to the presence of fewer elements. The reptilian calcaneum (ca) and distal tarsals (dt) 3 and 4 originate from the primary axis. The anterior proximal tarsal, however, may originate from the intermedium and other condensations (for a discussion of homologies, see Holmgren, 1933; Schaeffer, 1941; Mathur and Goel, 1976; Burke and Alberch, 1985; Shubin and Alberch, 1986). In *Hemiergis*, only one condensation was discernible directly anterior to the ca, but the embryonic connectivity of this element is ambiguous. Following traditional nomenclature, this element is here referred to as the astragalus (as). The order of tarsal appearance in all *Hemiergis* morphs examined is ca > dt4 > as > dt3.

Carpal and tarsal fusions. Cartilaginous fusions were frequently observed in the fore- and hind limbs of *Hemiergis*. In the forelimb, most *H. quadrilineata* (2/2) and *H. initialis* (5/5) specimens exhibited a fused dc4 and dc5 (“dc4+5”) at, or just before, parturition (Table 1). Fusion (or perhaps nonsegmentation) of the ue and c was also observed in a single juvenile specimen (HQ-004) of *H. quadrilineata* (2/2). In the hind limb, cartilaginous fusion of the as and ca begins in all morphs between stages 33 and 34 (Fig. 5), yielding a single “tarsale proximale” (Sewertzoff, 1931: 135) by stage 34–36.

Digit condensations: shared developmental configurations. Metacarpal and metatarsal 4—the first digit elements to appear in the fore- and hind limb, respectively—invariably condense along the

primary axis in amniotes (Burke and Alberch, 1985; Shubin and Alberch, 1986; Müller and Alberch, 1990). The axis of digital development then curves anteriorly and sequentially yields digits III and II. Digit V appears temporally after digit III but before digit II in *Hemiergis* (Fig. 4).

Phalanges are added to each digit in *Hemiergis* in the order of appearance of their supporting metacarpals or metatarsals. Hence, the first phalanx to appear in each limb is the proximal phalanx of digit IV (IV-1) (Fig. 6), yielding a phalangeal formula of X-0-0-1-0 (where “X” denotes absence of a digit and “0” indicates a metapodial but no phalanges). The addition of phalanx IV-1 to both sets of limbs during stage 33 marks the final common configuration in the development of the four morphs. *Hemiergis initialis* (5/5) was not represented by specimens with this specific morphology but, based on digit configurations (and relative Alcian blue staining intensities) of stage 33 and 34 embryos, this species likely also passes through a configuration with four metacarpals/metatarsals and one phalanx on digit IV.

At this final common phase of limb skeletal morphology, *Hemiergis* embryos are similar externally as well: all show similar degrees of development of the lower jaw, eyes, endolymphatic sacs, and (closed) branchial slits (Fig. 7). The limbs, too, are similar in external view and appear to contain the early condensations of four digits in both fore- and hind limbs; this similarity is especially pronounced between the two *H. peronii* (3/3 and 4/4) morphs (Fig. 8).

Phalangeal condensations: five-digit sequence. Following the above series of shared configurations, the limb skeletal developmental sequence of *Hemiergis initialis* (5/5) diverges from those of *H. peronii* (3/3 and 4/4) and *H. quadrilineata* (2/2) during stage 34. In addition to a divergence in skeletal developmental sequences, the overall shapes of the limb paddles differ between the four morphs as well (Fig. 9). At stage 34, *H. initialis* (5/5) adds mc1 and mt1, elements completely lacking in the other taxa (Fig. 10). Phalangeal addition also continues across all digits—not one digit at a time—until the adult configuration (2-3-4-4-3) is reached in both sets of limbs. Although *H. initialis* has five digits, its phalangeal configuration is slightly reduced from the primitive squamate morphology: on both the hands and feet, digit IV has only four phalanges, compared to the five present in the primitive squamate configuration (Figs. 1, 2D). Likewise, pedal digit V is missing a phalanx for an adult total of three.

Four-, three-, and two-digit sequences. The following results focus upon the forelimbs of *Hemiergis quadrilineata* (2/2) and *H. peronii* (3/3 and 4/4) (Fig. 11A), but hind limb sequences are nearly identical (Fig. 11B). After a shared digit configuration with single phalanges on digits III and IV, the two-, three-, and four-digit developmental sequences di-

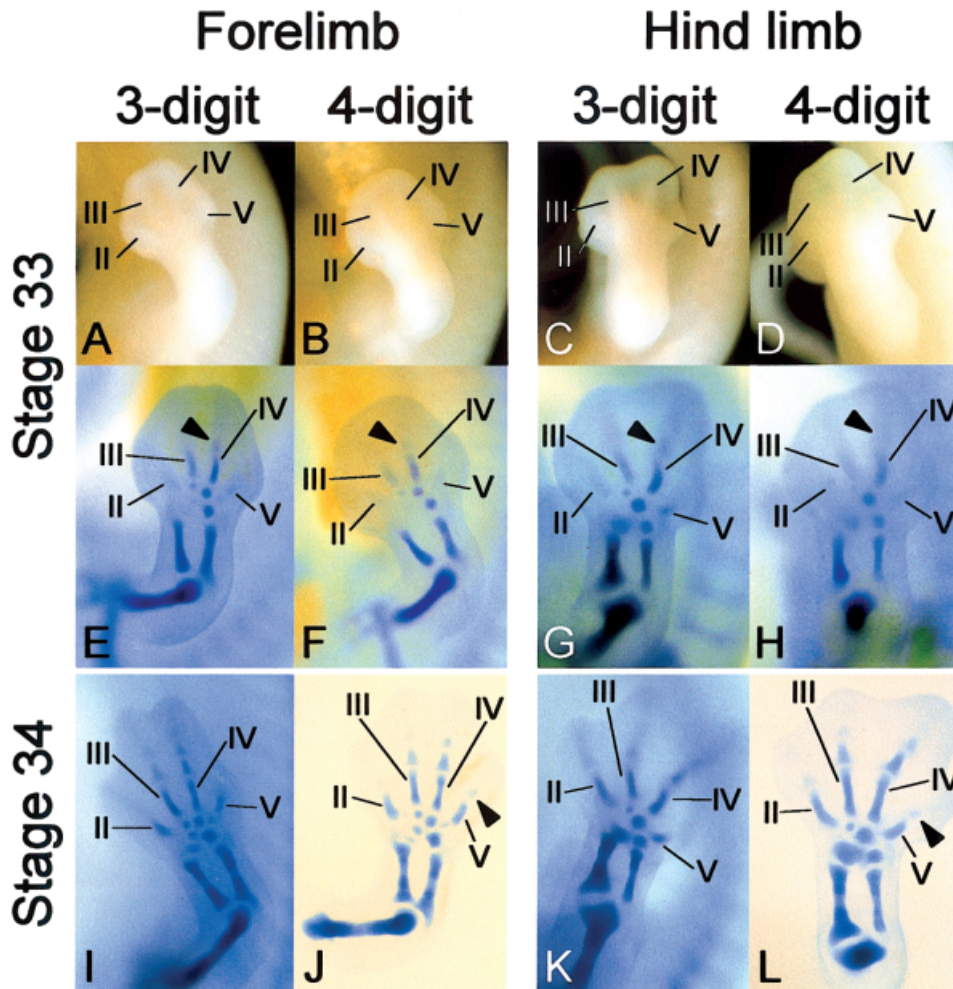


Fig. 8. Morphological divergence of three- and four-digit morphs of *Hemiergis peronii*. In all panels, digits II–V are indicated by roman numerals; anterior is to the left and distal is up. All limbs are shown in dorsal view. **A–D**: Stage 33 limbs of (**A,C**) *H. peronii* (3/3) and (**B,D**) *H. peronii* (4/4). At this stage, four digital condensations are visible externally in both the forelimbs (**A,B**) and hind limbs (**C,D**) of each morph. **E,F**: Forelimbs cleared and stained for cartilage verify four metacarpal condensations at this stage, with a single phalanx (arrowhead) over digit IV in each limb. **G,H**: Cleared and stained hind limbs reveal a similar condensation patterns. **I–L**: At stage 34, the proximal phalanx of digit V (arrowheads) appears in the four-digit morph (**J,L**), but not in the three-digit morph (**I,K**).

verge (Fig. 11; also see Fig. 8I–L). *Hemiergis peronii* (4/4) adds phalanges to the second, fourth, and fifth digits to bring the phalangeal formula to X-1-1-2-1. The proximal phalanges of digits II and V are added last to achieve this configuration, with IV-2 added slightly earlier. Phalanges subsequently condense proximally to distally across all four metacarpals and metatarsals until the adult phalangeal configuration of X-3-4-5-3 is attained.

After the shared X-0-0-1-0 configuration, digit V is excluded from the skeletal developmental program of *Hemiergis peronii* (3/3), and digits II and V from *H. quadrilineata* (2/2). *Hemiergis peronii* (3/3) adds phalanges across digits II, III, IV until the adult formula of X-3-4-5-0 is reached. *Hemiergis quadrilineata* (2/2) not only has a reduced number of digits, but also fewer phalanges than expected for digits III and IV: relative to the primitive squamate condition, single phalanges are lost from the two remaining digits for an adult formula of X-0-3-4-0.

Ossification of the Limb Skeletons

Stylopod and zeugopod. Whole-mount staining detects ossification of the stylopod element between

stages 36–38 in all morphs, followed shortly thereafter by zeugopod ossification (Tables 1, 2). The relative intensity and extent of staining indicate that the anterior zeugopod elements (forelimb: radius, hind limb: tibia) ossify before the posterior ones. However, serial sections of stage 33 and 34 embryonic limbs suggest that ossification begins earlier than is detectable by whole-mount alizarin staining. In *Hemiergis peronii* (4/4), for example, early perichondral ossification is observed in the humerus of a stage 33 forelimb (MCZ A38073) and the femur, tibia, and fibula of an *H. peronii* (3/3) stage 34 limb (MCZ A38117).

Secondary centers of ossification form in the long bone epiphyses postnatally, beginning with the stylopod elements (Table 2). In specimens that have secondary centers in only one zeugopod element (HQ-003, HQ-005, HP-005, HP-006, HI-006), the posterior element always dominates.

Carpals and tarsals. Carpal and tarsal ossification in *Hemiergis* also begins postnatally and carpal ossification lags behind the tarsus (also noted in other lizards by Rieppel, 1992b, 1993a, 1994a). In general, carpal ossification begins on the posterior

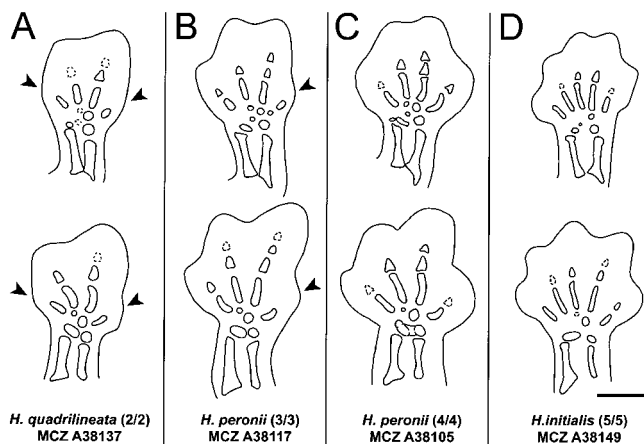


Fig. 9. Stage 34 *Hemiergis* limb paddles and skeletal condensations. **A–D**: Forelimbs are in the top row, hind limbs in the bottom row; anterior is to the left and distal is up. By this stage, the different morphs have divergent paddle shapes and condensation patterns. **A**: Limbs of *H. quadrilineata* (2/2) based on MCZ A38137. **B**: Limbs of *H. peronii* (3/3) based on MCZ A38117. **C**: Limbs of *H. peronii* (4/4) based on MCZ A38105. **D**: Limbs of *H. initialis* (5/5) based on MCZ A38149. In **A** and **B**, metacarpals and metatarsals that will not support phalanges have less mesenchyme distal to them (arrowheads). Dashed outlines indicate precartilaginous elements. Scale bar (**D**) = 0.5 mm.

side of the limb with the ue and dc4, followed by the re (Table 4). Carpal ossification is variable, however, in juvenile *H. initialis* (5/5; $n = 10$). In specimens with ossified carpals ($n = 3$), two (HI-006, HI-101) showed partial ossification of the re with no ossification or cartilage hypertrophy in other carpals. A third specimen (HI-002) showed dominance of the posterior carpals with an ossified ue and hypertrophied dc4 portion of dc4+5. A fourth (HI-105) showed no carpal ossification but hypertrophy of the ue and dc4.

In the forelimbs of five *Hemiergis quadrilineata* (2/2) neonates, the portion of dc4+5 proximal to mc4 stained with alizarin red while the portion supporting mc5 did not (Fig. 12A). A larger specimen (HQ-101), however, revealed separate ossification centers in each of these distal carpals (Fig. 12B). Hence, these elements ossified separately, despite having developed from a single cartilage (also observed in the salamander *Thorius*; J. Hanken, pers. commun.).

Tarsal ossification sequences are consistent between different *Hemiergis* morphs and occur in the following order: as > ca > dt4 > dt3. Although the as and ca fuse as cartilages, these two elements ossify separately (Sewertzoff, 1908; Mathur and Goel, 1976; Rieppel, 1992a, b). Ossification of the as begins in a similar position to its initial site of chondrogenesis, just distal to the medial edge of the tibia (Fig. 12C). In several specimens, secondary centers of ossification formed in the as, ca, and dt4 as the primary ossifications approached the edges of their respective cartilages (Fig. 12D). (For a discussion of

secondary ossification centers in lizard tarsals, see Dollo, 1884, and Mathur and Goel, 1976.)

Digits. Digit ossification begins late in embryogenesis in all four morphs (Tables 1, 2) and shaft ossification was complete or well underway in the metapodials and phalanges of all neonates examined. Early signs of cartilage hypertrophy appeared first in the hind limb, beginning with the metatarsals (mt) in the order mt4 > mt3 > mt2 (> mt1 in *Hemiergis initialis*, 5/5). Metatarsal 5 cartilages hypertrophied and ossified last in all morphs. Phalangeal hypertrophy begins with IV-1, III-1, and IV-2, which also showed the most advanced ossification across taxa. In general, ossification of the remaining phalanges occurred from proximal to distal, although ungual phalanges stained more intensely with alizarin red than did penultimate ones in some late-stage embryos. The forelimbs of all morphs follow a similar ossification sequence, although bone formation lags slightly behind the hind limb. Secondary centers of ossification appear in the metacarpals and metatarsals postnatally.

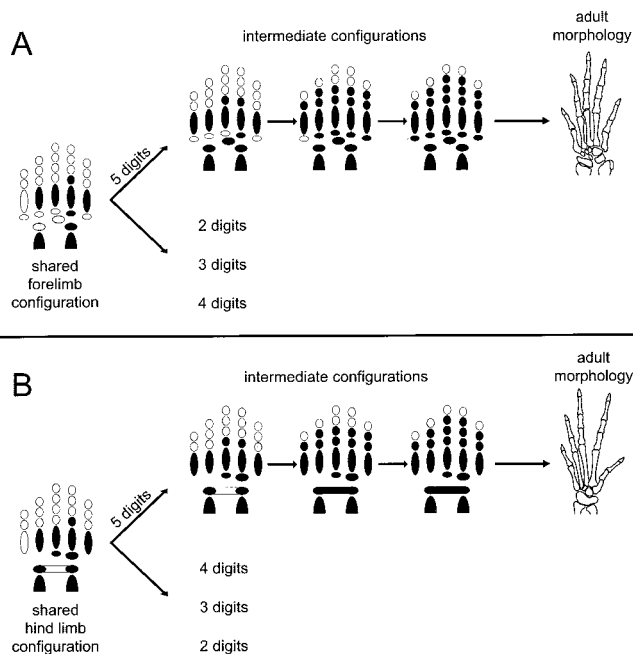


Fig. 10. Digit condensation sequence in *Hemiergis initialis* (5/5). Far left of **A,B**: Schematic diagrams of shared (**A**) fore- and (**B**) hind limb morphologies in all morphs of *Hemiergis*. Cartilaginous elements appear in black, precartilaginous in gray. Following this shared digit configuration, *H. initialis* (5/5) diverges from *H. peronii* (3/3 and 4/4) and *H. quadrilineata* (2/2). **A**: Forelimb condensation sequence. In *H. initialis* (5/5), metacarpal 1 is added last, along with phalanges over digits III, II, and V. Phalanges are subsequently added across all five digits until the adult phalangeal formula of 2-3-4-4-3 is attained. **B**: Hind limb condensation sequence. The pattern of phalangeal addition is similar to that of the forelimb. The adult hind limb phalangeal formula is identical to that of the forelimb.

Regression Analysis of Limb Lengths

Multiple regression analysis of limb lengths reveals significant differences among morphs ($P < 0.001$ for fore- and hind limb analyses) and these differences do not correlate in a simple linear fashion with digit numbers (Fig. 13). Relative to body length, *Hemiergis peronii* (4/4) has the longest fore- and hind limbs, followed by *H. peronii* (3/3). *Hemiergis initialis* (5/5) and *H. quadrilineata* (2/2) have relatively shorter limbs than both *H. peronii* (3/3 and 4/4) morphs, but their positions in the length

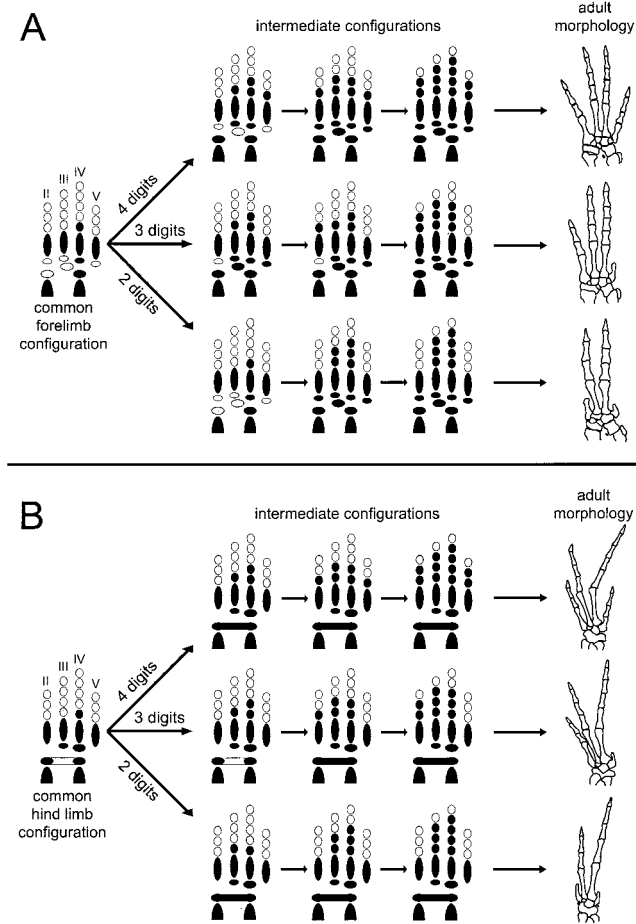


Fig. 11. Comparison of digit condensation sequences among two-, three-, and four-digit morphs of *Hemiergis*. Cartilaginous elements are indicated in black, precartilaginous in gray. **A:** In the forelimb, all three morphs share a common configuration with a phalangeal formula of X-0-1-1-0 (far left) before developmental trajectories diverge. Top row: In the four-digit *H. peronii*, phalanges are added proximally to distally across all four digits until the adult formula of X-2-3-4-3 is reached. Center row: In the three-digit *H. peronii*, phalanges are only added across digits II-IV; only a metacarpal remains in the digit V position. Bottom row: In the two-digit *H. quadrilineata*, phalanges are added only to the central digits, although four metacarpals are present. The adult phalangeal formula of *H. quadrilineata* (2/2) is X-0-3-4-0. **B:** Hind limb condensation sequences follow a similar pattern and adult phalangeal formulae are identical to those of the forelimbs for each morph.

TABLE 4. Carpal ossification sequences in *Hemiergis*

Species	Order of ossification
<i>H. quadrilineata</i> (2/2)	ue > dc4 > re ≥ (dc3, pis) > c > dc5 > dc2
<i>H. peronii</i> (3/3)	ue > dc4 > (re, c, dc5, dc3, pis) > dc2
<i>H. peronii</i> (4/4)	ue > dc4 > re > (c, dc3) > (dc5, dc2, pis)
<i>H. initialis</i> (5/5)	ue > dc4 ≥ re > (c, dc5, dc3, dc2, pis) > dc1

gradient vary between the forelimb and hind limb. In terms of digit number, the gradient of forelimb length (from longest to shortest) is $4 > 3 > 5 > 2$ (Table 5). In the hind limb, the gradient is $4 > 3 > 2 > 5$ (Table 6).

DISCUSSION

This study is the first to thoroughly document limb chondrogenesis sequences in any scincid lizard and the first comparison of limb ontogenies in a graded morphocline of any tetrapod. Previously, chondrogenesis sequences for only a few five-digit tetrapods had been reported (Sewertzoff, 1904, 1931; Steiner, 1922; Mathur and Goel, 1976) and not all of these ontogenetic sequences are complete. An advantage of studying limb chondrogenesis and reduction in *Hemiergis* is that closely related organisms—in this case, four morphs of three species within a single genus—are the focus. Moreover, one of these morphs has five digits, representing the putative primitive condition for the group (although phalangeal counts in *H. initialis*, 5/5, differ slightly from the primitive squamate configuration). Hence, studies of limb development in *Hemiergis* could provide insights into both general and derived patterns of lizard limb skeletal ontogeny.

The limb chondrogenesis sequence for *Hemiergis initialis* (5/5) resembles those described for other pentadactyl lizards. Importantly, truncations of this program at intermediate developmental stages would yield incomplete digits, not fewer complete ones. Hence, the adult limbs of *Hemiergis* with fewer than five digits do not result from simple heterochronic truncations of a five-digit (or any other) skeletal developmental program. Ossification sequences in *Hemiergis* resemble those of other lizards as well and all cartilaginous elements formed are eventually replaced by bone, thereby eliminating ossification failures as a source of limb reduction.

This investigation also identifies positional homologies between digits of different *Hemiergis* morphs. Morphological cues in adult *Hemiergis*, such as phalangeal formulae, can be misleading when assigning digit positional homologies. As discussed below, this developmental study confirms that digit I is indeed lost in *H. quadrilineata* (2/2) and *H. peronii* (3/3) and

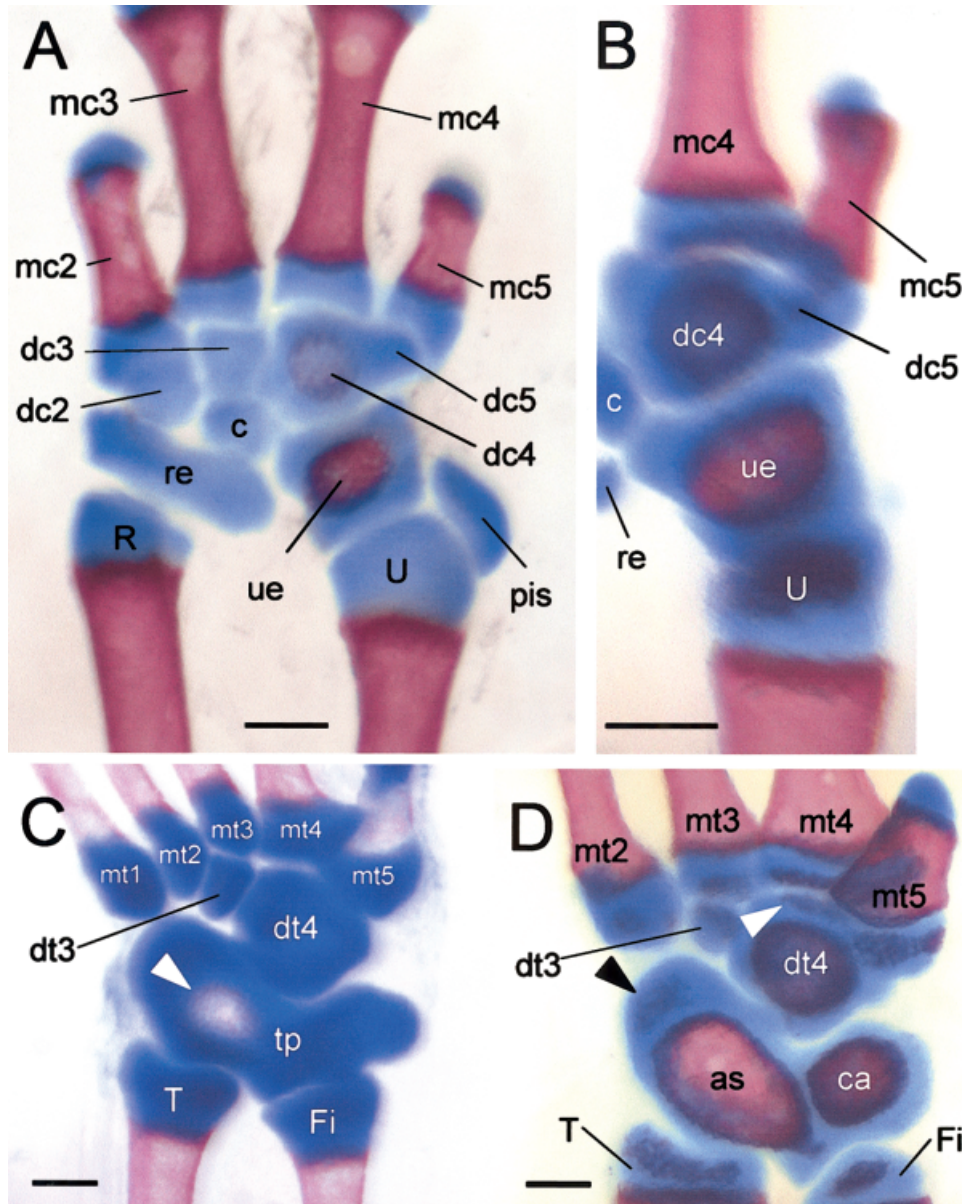


Fig. 12. Carpal and tarsal ossification in *Hemiergis*. **A,B**: Independent ossification of distal carpals 4 and 5 in *Hemiergis quadrilineata* (2/2) juveniles. **A**: Dorsal view of wrist of HQ-001 (SVL = 22.5 mm) cleared and stained for cartilage (blue) and bone (red). The earliest ossification center of distal carpal 4 + 5 (the cartilaginous fusion of distal carpals 4 and 5) is located directly proximal to metacarpal 4 and does not include cartilaginous distal carpal 5. **B**: Dorsal view of wrist of HQ-101 (SVL = 30 mm). In this larger specimen, ossification of distal carpal 5 begins adjacent to—but distinct from—distal carpal 4. **C,D**: Tarsal ossification in *Hemiergis* juveniles. **C**: Dorsal view of ankle of *H. initialis* (5/5; HI-105) showing early ossification of the astragalus (arrowhead) within the tarsale proximale, the cartilaginous fusion of the astragalus and calcaneum. **D**: Ventral view of ankle of *H. quadrilineata* (HQ-101) showing secondary ossification centers in the astragalus (black arrowhead) and distal tarsal 4 (white arrowhead) within the tarsale proximale. Additional secondary centers of ossification are visible in the proximal metacarpals and distal tibia and fibula. as, astragalus; c, centrale; ca, calcaneum; dc, distal carpal; dt, distal tarsal; Fe, femur; Fi, fibula; H, humerus; mc, metacarpal; mt, metatarsal; pis, pisiform; R, radius; re, radiale; T, tibia; tp, tarsale proximale; U, ulna. Scale bars = 0.1 mm.

4/4) and that at least some portion of digits II–V is retained. Interestingly, phalanges appear in digit V of several *H. peronii* (3/3) embryos (Table 1), representing an atavism or low-frequency polymorphism.

The functional significance of different digit configurations in *Hemiergis* is difficult to assess without controlled studies of locomotion. However, a regres-

sion analysis of limb and body lengths reveals that digit number is not linearly correlated with limb length. When coupled with behavioral observations of wild and captive *Hemiergis* and other reduced-limbed skinks (discussed below), these results permit limited generalizations about the functional correlation between digit number and limb length.

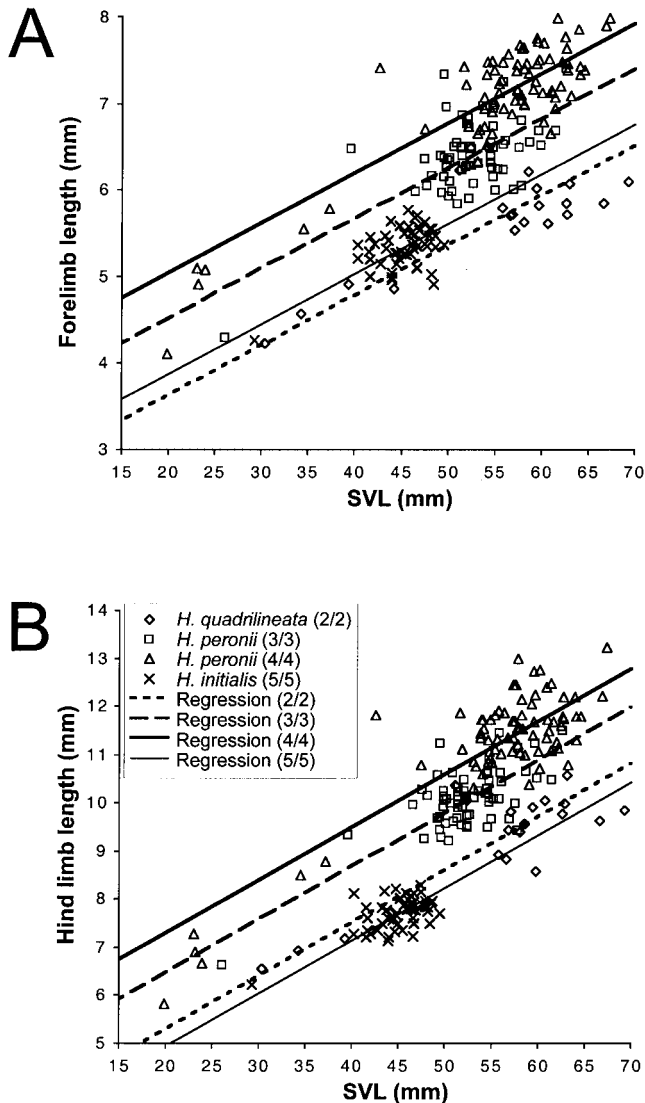


Fig. 13. Plots and regressions of limb lengths vs. SVL for *Hemiergis* (A) forelimbs and (B) hind limbs. A: At a given SVL, *H. peronii* (4/4) forelimbs are longer than those of *H. peronii* (3/3), *H. initialis* (5/5), and *H. quadrilineata* (2/2), respectively. Slope = 0.058 for forelimb regression lines. B: The two *H. peronii* morphs also have the longest hind limbs, but the relative lengths of *H. initialis* (5/5) and *H. quadrilineata* (2/2) limbs are reversed compared to the forelimb. Slope = 0.11 for hind limb regressions. See Tables 5 and 6 for regression statistics.

Digit Loss in *Hemiergis* Does Not Result From Simple Developmental Truncations of Chondrogenic Sequences

Despite differences in adult digit numbers, a similar mode of phalangeal condensation characterizes *Hemiergis* developmental sequences: phalanges are added from proximal to distal across all phalanx-bearing digits in all developmental sequences. This mode of digit assembly, shared by all known amniote sequences, limits the ways that digit loss in *Hemiergis* can be described. If digits formed one at a

time, for example, early truncations of an ancestral limb development program would produce fewer (but still complete) digits. In this hypothetical situation, the last digits to form in the ancestral program would be lost, but the remaining digits would maintain their ancestral morphologies with full complements of phalanges. For instance, if digit I in *H. initialis* (5/5) formed after digits II–IV were fully formed, then the loss of digit I in the two-, three-, and four-digit morphs could be explained by a truncation of the five-digit developmental program. However, this scenario was not observed in *Hemiergis*. Instead, a truncation of the *H. initialis* (5/5) sequence prior to the appearance of digit I would yield a phalangeal formula of X-0-0-1-0, which corresponds to an intermediate stage—not the adult stage—in the development of the other species. Hence, the loss of digit I in *H. quadrilineata* (2/2) or *H. peronii* (3/3 and 4/4) cannot be described as a truncation of a five-digit developmental sequence.

Likewise, the absence of digit V phalanges in the two- and three-digit morphs cannot be explained by a simple truncation of another developmental program. The limb skeleton developmental sequences of *Hemiergis quadrilineata* (2/2), *H. peronii* (3/3 and 4/4), and *H. initialis* (5/5) are similar until stage 33, after which *H. initialis* adds digit I (Fig. 10). By stage 34, *H. peronii* (4/4) adds phalanges to digit V, whereas *H. peronii* (3/3) and *H. quadrilineata* (2/2) do not (Fig. 11). If the four-digit developmental program were to be truncated at the latest stage when digit V has no phalanges, the resulting phalangeal formula would be X-0-1-1-0, not the three-digit adult formula of X-3-4-5-0, or the two-digit adult formula of X-0-3-4-0. Since the adult digital configurations of reduced-limbed *Hemiergis* are not represented in the developmental sequences of the five-digit (or any other) morph, these reductions cannot be explained as simple truncations. Furthermore, the adult configurations of neither the two-, three-, nor four-digit *Hemiergis* morphs are represented at any known intermediate stage in the development of any pentadactyl lizard.

A similar situation can also be observed in the scincid *Lerista*, whose species have between zero and five digits on both sets of limbs (Greer, 1987, 1990, 1991). Although limb development sequences have not been described for any *Lerista* species themselves, the reduced adult digit morphologies in *Lerista* do not correspond to intermediate developmental stages in any known ontogenetic sequences (Greer, 1991). Shubin and Alberch (1986) also describe a parallel example in the salamander *Proteus*, in which two-fingered and three-toed adult limbs do not correspond to a single developmental stage of any other salamander. Hence, the mode of digit loss observed in *Hemiergis* may characterize a diverse group of tetrapods.

Paradoxically, the one limb morphology in *Hemiergis* that could be explained by a simple trunca-

TABLE 5. Parameter estimates for *Hemiergis* forelimb length regressions ($R^2 = 0.87$)

	Coefficients	Standard error	<i>t</i> -Stat	<i>P</i> -value
Intercept	2.478209217	0.183326148	13.51	<0.0001
SVL	0.057609023	0.00306966	18.76	<0.0001
<i>H. peronii</i> (3/3)	0.887640912	0.082522663	10.75	<0.0001
<i>H. peronii</i> (4/4)	1.406038609	0.080796366	17.40	<0.0001
<i>H. initialis</i> (5/5)	0.25208638	0.090200352	2.79	0.0057

tion of an ancestral sequence occurs in the species with the most digits. *Hemiergis initialis* has five manual and pedal digits, but digits IV (both sets of limbs) and V (hind limb only) are each reduced by one phalanx relative to the ancestral squamate condition. These distal phalanges are among the last elements to form in a complete chondrogenic series and thus their absence in *H. initialis* may reflect a foreshortened developmental program.

In contrast to *Hemiergis* and *Lerista*, major trends of limb reduction in other lizards likely do represent truncations of ancestral sequences. For example, the adult morphologies of some species of the teiid *Bachia* (Presch, 1975) are paralleled by intermediate developmental stages of other lizards (Fig. 14). Unlike *Hemiergis* and *Lerista*, adult *Bachia* forelimb digit configurations closely resemble early ontogenetic stages of other squamates. The *Bachia* example is significant because it contrasts with the dominant mode of digit loss observed in *Hemiergis* and *Lerista*, which is thus not generalized for all cases of limb reduction in lizards.

Digit Loss in *Hemiergis* Does Not Result From Truncations of Ossification Sequences

Rieppel (1992b) identifies two modes of skeletal reduction in vertebrates: 1) failure to “preform” an element in cartilage, and 2) failure to ossify. Since the sequence of limb ossification in squamates does not necessarily follow the order of cartilage condensation, elements lost by a truncation of an ossification sequence may not be the same elements lost by a truncation of cartilage formation. For example, the loss of carpals in *Lerista* (for which no ossification sequences are known) corresponds to truncations of a known pentadactyl lizard ossification sequence, rather than to a truncation of a pentadactyl chondrogenesis sequence (Rieppel, 1992b). That is, carpals lost in reduced-limbed *Lerista* are those that

ossify last in pentadactyl lizards, not those that are formed in cartilage last. This decoupling of chondrogenesis and ossification sequences may extend to the digits as well (Rieppel, 1992a, b, 1993a, 1994a, b). Thus, truncations of osteogenesis may generate limb morphologies not predicted by truncations of chondrogenesis.

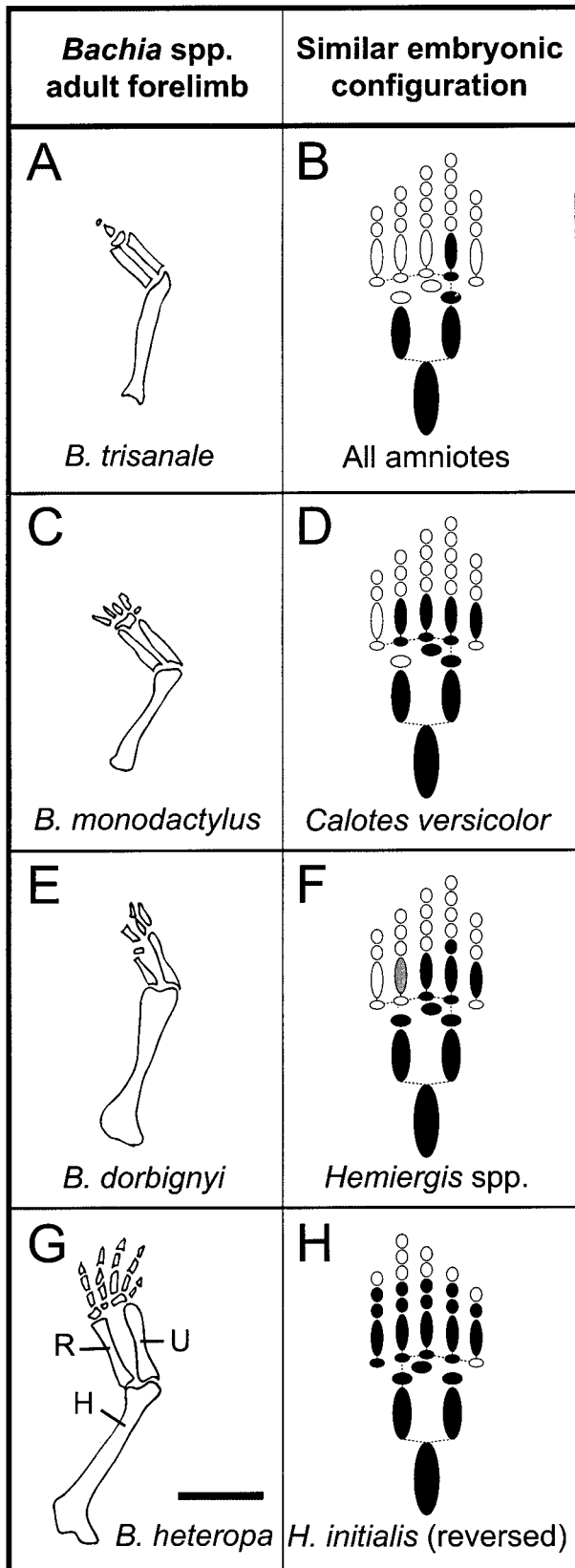
In *Hemiergis*, however, ossification sequences do not lend any predictive insight regarding the order or mode of digit loss. All skeletal elements that are preformed in cartilage eventually ossify and elements are not lost in the reverse order of ossification. If ossification truncations were responsible for missing digits, the expected order of digit loss would be V > I > II > III > IV. Instead, digit I is the most frequently missing, followed by digit V and digit II (I > V > II), the typical pattern of phylogenetic digit loss in amniotes (Morse, 1872; Sewertzoff, 1931; Raynaud, 1987; Greer, 1987, 1989, 1990, 1991). Hence, rather than suggest a role for ossification failures in the evolution of digit loss, this reduction pattern reaffirms the independence of limb chondrogenesis and ossification sequences in lizards (Rieppel, 1992b, 1994a).

Evolutionary Digit Loss and the Terminology of Heterochrony

Heterochrony may be divided into two principal categories, both of which describe morphological changes relative to an ancestral state (Alberch et al., 1979). In the first category, peramorphosis, a descendant morphology extends beyond the ancestor by accelerating, prolonging, or precociously activating the ancestral developmental trajectory. Conversely, pedomorphosis describes a descendant adult morphology that resembles that of a juvenile (or embryonic) stage of the ancestor. Pedomorphosis results from a developmental trajectory that is

TABLE 6. Parameter estimates for *Hemiergis* hind limb length regressions ($R^2 = 0.90$)

	Coefficients	Standard Error	<i>t</i> -Stat	<i>P</i> -value
Intercept	3.092510584	0.305812335	10.11	<0.0001
SVL	0.110382679	0.0051206	21.55	<0.0001
<i>H. peronii</i> (3/3)	1.182258358	0.137658749	8.58	<0.0001
<i>H. peronii</i> (4/4)	1.992376985	0.134779057	14.78	<0.0001
<i>H. initialis</i> (5/5)	-0.35834317	0.150466153	-2.38	0.018



slowed down (neoteny), truncated (progenesis), or delayed in its onset (postdisplacement).

At the level of the entire limb skeletal development program in *Hemiergis*, digit loss cannot be regarded as the result of progenesis: the overall timing of the likely ancestral chondrogenesis and osteogenesis sequences are not modified. However, at the level of specific events within these sequences, truncations or deletions may indeed result in an absence of metacarpals and phalanges. For instance, a lack of anterior digital arch bifurcation may lead to an absence of digit I in *H. peronii* (3/3 and 4/4) and *H. quadrilineata* (2/2), and nonsegmentation of digit V in *H. peronii* (3/3) and *H. quadrilineata* may produce a metacarpal without phalanges. However, identifying these examples at the level of the digital arch and individual digit segmentation as pedomorphic heterochronic events is of questionable utility; with respect to autopod development, digits are integral parts—not terminal events—of a larger unit (see Shapiro and Carl, 2001, for extended discussion). Moreover, digit quantity in tetrapods may be largely determined by processes and events that precede digit development itself, such as cell proliferation of the limb mesenchyme (Alberch and Gale, 1983; Alberch, 1985; Raynaud, 1986, 1989, 1991; Raynaud and Clergue-Gazeau, 1986). Hence, the evaluation of evolutionary changes in developmental timing depends largely on the level of organization examined (Raff and Wray, 1989). By ascribing heterochrony to any developmental process, or to a component of a larger process, essentially all evolutionary phenotypic changes can be attributed to heterochrony. Consequently, “heterochrony” becomes synonymous with morphological change and thus

Fig. 14. Adult forelimb morphologies of *Bachia* spp. (A,C,E,G) closely resemble embryonic configurations of other lizards (B,D,F,H). Anterior is to the left and distal is up in all panels. Blackened shapes in diagrams of embryonic morphologies represent cartilaginous, precartilaginous, and mesenchymatous condensations. Dashed lines in B,D,F,H indicate probable developmental connectivity between condensations. A: Forelimb of *B. trisanale*. From proximal to distal, the elements of the limb likely include the humerus, radius and ulna, ulnare (possibly fused with the radiale), fourth distal tarsal, and fourth metacarpal. B: These elements should be present in all amniotes with at least one digit, according to models of limb morphogenesis (Burke and Alberch, 1985; Shubin and Alberch, 1986). C,D: The adult forelimb of *B. monodactylus* and an embryonic limb configuration in *Calotes versicolor* (Mathur and Goel, 1976) both have four metacarpals distal to the carpals. E,F: The adult forelimb of *B. dorbignyi*, with three metacarpals and a single phalanx over digit IV, corresponds closely to an embryonic configuration in *Hemiergis*; however, *Hemiergis* has an additional metacarpal (metacarpal 2, shaded) when the first phalanx appears. G,H: The adult forelimb of *B. heteropa*, with two phalanges over digits I–IV and a single phalanx over digit V, resembles an embryonic configuration of *H. initialis* with a reversed anteroposterior axis. The *H. initialis* diagram in H is reversed so that digit V is on the far left, digit I on the far right. *Bachia* figures redrawn from Presch (1975). Scale bar (G) = 1 mm for all *Bachia* drawings.

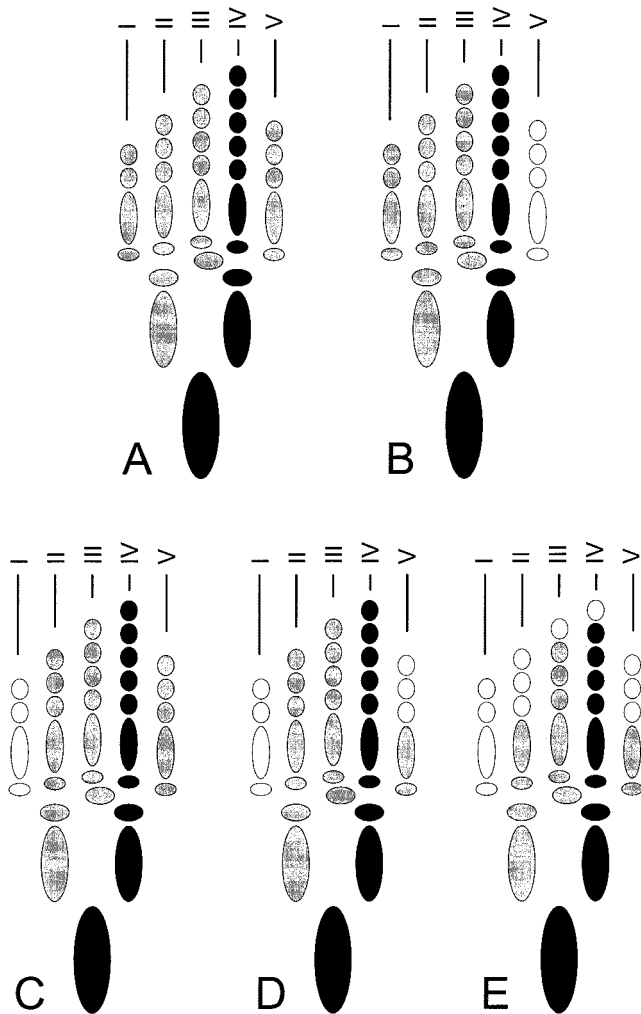


Fig. 15. Positional homology of *Hemiergis* digits. A–E: Schematic representations of complete and reduced forelimb configurations. Digits are numbered from anterior (digit I) to posterior (digit V). Shading indicates presence of an element; elements shaded black lie along the primary axis. A: Generalized lizard forelimb with a full complement of digits and phalanges based on the skink *Glaphyromorphus gracilipes*. B: Hypothetical four-digit configuration resulting from the loss of digit V, the digit posterior to the primary axis. Digit IV—the primary axis digit—becomes the most posterior digit. C: Four-digit configuration resulting from the complete loss of the anterior digit (digit I), as observed in *H. peronii* (4/4). D: Configuration with three complete digits and a metacarpal posterior to the primary axis (digit V), as observed in *H. peronii* (3/3). E: Configuration with two digits bearing phalanges, and two metacarpals without phalanges, as observed in *H. quadrilineata* (2/2). The two central digits in E have phalangeal formulae resembling digits II and III in A, but develop in the positions of digits III and IV, respectively.

loses any meaning of its own (Wake, 1996; Zelditch and Fink, 1996; Rice, 1997; Gould, 2000).

Mesopodial Chondrogenesis Sequences and Fusions

Mesopodial chondrogenesis and ossification sequences in *Hemiergis* are similar to those previously

described for other lizards (e.g., Mathur and Goel, 1976; Rieppel, 1992a,b, 1993b, 1994b). Nevertheless, the different morphs examined exhibit slight differences in the order of carpal chondrogenesis, but this variation is likely not attributable to any significant developmental reprogramming. Instead, these differences are likely the result either of slight variations in condensation timing away from the primary axis and/or a bias introduced by the relatively small number of specimens available for study.

All carpal chondrogenic sequences are consistent with the same pattern of branching and condensation. The ue yields dc4 and the ce, and thus it necessarily precedes the others. Distal carpals 3, 2, and 1 (*Hemiergis initialis*, 5/5, only) lie along the digital arch and follow condensation of dc4; distal carpal 5 is separate from the primary axis and develops independently (Burke and Alberch, 1985; Shubin and Alberch, 1986). The re originates from the anterior side of the limb and thus its position in the condensation sequence is also likely independent of the other carpals.

Two cartilaginous fusions occurred consistently in the carpals and tarsals of *Hemiergis*: dc4 and 5 in *H. initialis* (5/5) and *H. quadrilineata* (2/2), and the as and ca in all four morphs. In both cases, these elements first appear as distinct and separate cartilages. Interestingly, despite fusing well before ossification begins, each element develops a separate center of endochondral ossification. Hence, despite fusion into a single cartilage, these elements appear to retain their initial identities in the context of ossification.

Positional Homologies of Digits in *Hemiergis*

Positional homologies of digits can only be confirmed by studies of developmental morphology because postnatal digit numbers may not be informative. In pentadactyl lizards such as *Hemiergis initialis*, the positional homologies of digits are easy to establish with other amniotes. In limbs with fewer than five digits, however, the identity of the digit originating from the primary axis—digit IV—may become obscured, thus making it difficult to determine the positional identity of other digits. In a four-digit limb, for example, condensation sequence and position data can reveal which digit is “lost.” If the digit posterior to the primary axis fails to form, then digit V is missing (Fig. 15B). If the postaxis digit forms but only two digits condense anterior to the primary axis, then digit I is missing, as is the case in *H. peronii* (4/4) (Fig. 15C).

Embryological data are also important because postnatal morphological cues can be misleading. Among reduced-limbed *Hemiergis* morphs, for example, digit positional homologies with the primitive squamate condition are supported by evidence

from adult phalangeal formulae in the two *H. peronii* (3/3 and 4/4) morphs, but not in *H. quadrilineata* (2/2) (Fig. 15). The anterior three digits of both *H. peronii* morphs have the phalangeal formulae 3-4-5, which correspond to the primitive squamate configurations for digits II, III, and IV (Romer, 1956). The digits bearing phalanges in *H. quadrilineata* (2/2), however, are not as easy to homologize using phalangeal formulae only. These digits bear three and four phalanges, which correspond to digits II and III, respectively, of the primitive squamate configuration. Embryological data, however, contradict this interpretation because the digit with four phalanges is the primary axis digit, which is thus homologous to the ancestral digit IV with a reduced phalangeal count. Similar observations (Sewertzoff, 1931; Raynaud, 1987) confirm the interpretation of the three forelimb digits of *Chalcides chalcides* as II, III, and IV, rather than I, II, and III, as originally proposed on the basis of adult morphology (Steiner and Anders, 1946).

Phalangeal formulae alone can be misleading in assigning digital homology in *Hemiergus*, but other adult morphological cues may be useful. For example, mt5 of all morphs, including those with no phalanges on digit V, retain the "hooked" morphology characteristic of all lepidosaurs (Carroll, 1988; Gauthier et al., 1988; Fig. 2). Furthermore, all morphs share the lepidosaurian trait of mc3 being at least as long as mc4 (Gauthier et al., 1988) and mt4 has the broadest proximal epiphysis. These characters suggest that not only are the *positions*—the locations relative to the primary axis—of the digits in *Hemiergus* homologous, but the *identities*—the morphologies of constituent bones—of the digits are as well, despite differences in phalangeal count. Hence, digits (or entire complements of phalanges) are lost in the order I > V > II in *Hemiergus*, thereby reaffirming the evolutionary stability of digits III and IV in squamates. This order of loss could have been predicted based on adult morphology, but, importantly, it could only be verified through developmental observations.

The general pattern of digit loss and reduction observed in *Hemiergus* is seen in a wide variety of other tetrapod groups as well (Morse, 1872; Romer, 1966; M.D. Shapiro and N.H. Shubin, unpubl. obs.). Theropod dinosaur forelimbs are a possible exception to this generalization and the problem of theropod (and avian) manual digit identity—including the possibility of evolutionary frameshifts in digital identities—has been debated extensively in the literature (for recent analyses, see Burke and Feduccia, 1997; Wagner and Gauthier, 1999).

Atavistic Digit V in *Hemiergus peronii* (3/3)

Supernumerary digits have long been recognized in modern horses (Marsh, 1892; Ewart, 1894) and in some lizards (e.g., Papenfuss, 1982). Some of these

occurrences meet the criteria for an atavism, defined by Hall (1984: 89) as "the reappearance of a lost character (morphology or behavior) typical of remote ancestors and not seen in the parents or recent ancestors of the organisms displaying the atavistic character." Likewise, the limbs of *Hemiergus peronii* (3/3) typically lack phalanges on digit V, but this population has the developmental potential to form this digit. If we assume that a complete digit V was present in the ancestor of *H. peronii* (3/3), the reappearance of digit V phalanges in several specimens also meets the criteria for an atavism.

In three embryonic specimens of *Hemiergus peronii* (3/3), one (MCZ A10476) or more (MCZ A10497, MCZ A10478B) manus digit V phalanges are present. Choquenot and Greer (1989) also noted the appearance of phalanx V-1 in both limbs in approximately 5% of adult *H. peronii* (3/3) specimens they examined. All three embryos with extra phalanges in this study were obtained near the same locality at 33°42' N, 115°53' E, a site that also yielded several adult specimens with four fingers and three toes, but never four toes. This locality was among the easternmost collection areas for the three-digit population, approaching the range of the four-digit population.

While the three- and four-digit populations of *Hemiergus peronii* appear to be parapatric (Choquenot and Greer, 1989), it is conceivable that hybrid zones may occur along shared range borders. However, it is probably too simplistic to assume that a mating between an individual from each population would yield offspring with an intermediate morphology. Indeed, within a single adult specimen (MCZ A10478) with four fingers and three toes (4/3), only two of four embryos harvested had four fingers; the presence of a complete digit V was observed at same ratio in one other 3/3 specimen (MCZ A10476, which also had the aforementioned embryo with V-1 only). Both embryos of MCZ A10497, another female with three digits on each limb, had the 4/3 configuration.

The uncommon—but not rare—occurrence of four-fingered, three-toed *Hemiergus peronii* at this site deserves more rigorous studies of biogeography and population genetics to identify potential zones of gene flow between these populations. Such studies could also test the possibility that the 4/3 configuration is a low-frequency polymorphism in this population rather than a developmental phenomenon. Interestingly, the extra phalanges in the forelimb of these *H. peronii* contradict the evolutionary pattern of limb reduction in other skinks such as *Lerista*, in which the phalanges of the manus are lost at a higher rate than the pes (Choquenot and Greer, 1989).

Limb Length: Possible Functional Correlates

Limb element loss and limb shortening occur extensively in squamate evolution (see Greer, 1990;

Gans, 1975, for reviews) but aspects of their co-occurrence have not been adequately documented. Moreover, the functional morphology of completely limbless squamates has drawn considerable attention, but little is known about the functional morphology of lizards with shortened or incomplete, but not absent, limbs. Gans (1975) highlights the evolution of trunk elongation relative to body diameter as a prerequisite for limb shortening, a trend supported to a more limited extent by Greer (1991) and Carroll (1996). Body elongation, especially in small forms (Carroll, 1996), may allow some organisms to exploit new microhabitats (crevices, leaf litter, etc.) that larger animals cannot access.

Previous analyses of limb reduction and body elongation compare vertebral and limb bone quantity within *Lerista* (Greer, 1987) and *Hemiergis* (Choquenot and Greer, 1989). The former study suggests that limb element quantity is negatively correlated with vertebral quantity, but no significant correlation was found in the latter. Hence, *Lerista* species with more vertebrae tend to have fewer limb bones—including fewer digits, since distal bones are the first to be lost—and vice versa, while the same correlation does not necessarily hold true for *Hemiergis*.

The present study compares *Hemiergis* morphs using a set of parameters that are arguably more germane to function than those used in previous analyses. Rather than comparing numbers of trunk and limb segments, the linear dimensions of the structures actually interacting with the locomotor substrate are considered. The regression analysis presented here demonstrates that limb lengths vary significantly between the different *Hemiergis* morphs with respect to a standardized SVL, but these differences do not correlate directly with digit quantity. Among the most closely related morphs—*H. quadrilineata* (2/2) and *H. peronii* (3/3 and 4/4) (J. Reichert and T. Reeder, pers. commun.)—the correlation between digit number and limb length is direct in both sets of limbs: $4 > 3 > 2$. The pentadactyl *H. initialis*, the morph with the most digits and highest phalangeal count, has the shortest hind limbs and the second-shortest forelimbs. Hence, the complete gradients of limb lengths in terms of digit numbers are: $4 > 3 > 5 > 2$ for the forelimb, and $4 > 3 > 2 > 5$ for the hind limb.

These results are significant because they provide clues about the functional correlates of digit loss in lizards. *Hemiergis* spp. are small, semifossorial lizards with elongate bodies and shortened limbs. All morphs use their limbs to varying degrees when above the surface and adopt lateral undulation with limbs adpressed to the trunk (forelimbs) or tail (hind limbs) when moving in litter or during an escape response (Choquenot and Greer, 1989; pers. obs.). All morphs are also capable of shallow burrowing under large objects (e.g., logs) and in loose substrate, but *H. quadrilineata* (2/2) burrows extensively in

captivity and in the wild (pers. obs.). Captive adult *H. quadrilineata* were typically found at the bottom of their enclosures, having burrowed under several centimeters of wet sand, whereas *H. peronii* (3/3 and 4/4) and *H. initialis* (5/5) rarely ventured more than their dorsoventral depth below the surface. Greer (1989) describes similar observations of the sand-swimmer *Lerista*, in which species with more than three forelimb digits prefer surface-based foraging, whereas species with three or fewer forelimb digits prefer subsurface-based foraging. Hence, the burrowing habits of the two-digit *H. quadrilineata* suggest that this trend may be generalized for both sand- and litter/humus-swimming skinks (also see Gans, 1975, for a detailed discussion of limbless and reduced-limbed locomotion).

The pentadactyl *Hemiergis initialis*, the morph with the most digits and highest phalangeal count, has the shortest hind limbs and the second-shortest forelimbs. These discrepancies may betray a phylogenetic bias (*H. initialis* lies outside the *H. quadrilineata* – *H. peronii* clade; J. Reichert and T. Reeder, pers. commun.), but several habitat differences may be factors as well. Like *H. quadrilineata* (2/2) and *H. peronii* (3/3 and 4/4), *H. initialis* (5/5) is a litter-dweller, but it lives in areas dominated by lateritic soils that often contain large granite outcrops, whereas the other morphs live on relatively smoother sand plains. Perhaps a greater number of digits and limbs of subequal length in *H. initialis* (5/5) confers a functional advantage in navigating rockier terrain and signal a lesser emphasis on undulatory locomotion than in other *Hemiergis* morphs. The shorter tail—and thus reduced surface for undulatory locomotion—of *H. initialis* compared to its congeners (Storr et al., 1999) would seem to support this hypothesis as well. Detailed comparative studies of ecology and locomotion in *Hemiergis* will be necessary to test this and other functional hypotheses directly (see Kohlsdorf et al., 2001, for an example in anoline lizards).

On a developmental level, the incongruence between limb length and digit number suggests that the regulation of linear growth of the limb is independent of the regulation of digit loss. Hence, developmental mechanisms that limit digit formation do not necessarily limit overall limb length in postnatal *Hemiergis*.

CONCLUSIONS

This study is the first to detail chondrogenesis and osteogenesis sequences of the limb skeleton in scincid lizards and the first comparative developmental study of graded limb reduction in natural populations of any tetrapod. *Hemiergis* limbs with fewer than five digits do not result from a simple truncation of a five-digit (or any other) developmental program. Rather than yield fewer numbers of complete digits, heterochronic truncations of a five-digit pro-

gram at intermediate developmental stages would yield incomplete digits. Comparative analyses of two-, three-, four-, and five-digit developmental sequences revealed little, if anything, about heterochrony as an evolutionary *mechanism* of limb reduction; the developmental and adult morphologies in reduced-limbed *Hemiergus* are neither predicted nor explained by heterochrony.

Digit loss in *Hemiergus* occurs in the order $I > V > II$. While this order of loss is implied by some adult morphological cues, digit positional identity can only be verified through developmental studies. *Hemiergus peronii* (3/3) normally lacks a complete manus digit V, but several specimens from one locality bear digit V phalanges. This "reappearance" of skeletal elements may represent an atavism or low-frequency polymorphism and morphological variation within this population deserves additional attention.

In postnatal *Hemiergus*, digit number and relative limb length do not correlate in a simple linear fashion. In terms of digit number, relative forelimb length follows the order $4 > 3 > 5 > 2$, whereas relative hind limb length follows the order $4 > 3 > 2 > 5$. Limb size and element reduction may correlate with substrate conditions and burrowing behavior.

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